THE LIFE CYCLE AND TAXONOMY OF *PARADOXIA MULTISETA* SVIR. (CHLOROPHYCEAE, CHLOROCOCCALES), AND RELATED TAXA

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ABSTRACT. — The coenobia of *Paradoxia multiseta* form within the mother cell wall and not by aggregation of free swimming cells. The release of daughter cells follows dissolution of the mother cell wall. Mature vegetative cells are identical, whether formed from coenobia or free, single cells. Both zoospores and young vegetative cells have two contractile vacuoles. A stigma is found in zoospores as well as in vegetative cells. Cysts are observed. Unlike cells developing from zoospores, autospores lack bristles and appendages and have less pronounced heteropolarity.

Ankyra paradoxioides is transferred to *Paradoxia* as *P. paradoxioides* (Cirik) Hegew. et Reymond. Clearer distinctions have been observed between *Paradoxia* and *Ankyra* ability to produce coenobia, behavior of mother cell wall at sporulation, presence of bristles, fine structure of the pyrenoid and appearance of cell pole are the differentiating criteria. The identity of *Actinastrum guttula* Playfair (= *Paradoxia multiseta* ?) is discussed.

RÉSUMÉ. — Les cénobes de *Paradoxia multiseta* se forment dans la cellule mère et non par association de zoospores nageant librement. La libération des cellules filles se fait par gélification de la paroi de la cellule mère (et non par une déchirure). Qu'elles soient indépendantes ou faisant partie d'un cénobe, les cellules végétatives adultes ont une forme identique. Les zoospores ainsi que les jeunes cellules végétatives ont deux vacuoles pulsatiles. On observe un stigma aussi bien chez les zoospores que chez les cellules végétatives. Des kystes peuvent être formés lors du cycle vital. Contrairement aux cellules se développant à partir de zoospores, les autospores ne développent que très tardivement les soies et les appendices, et leur dissymétrie antério-postérieure est moins prononcée.

Ankyra paradoxioides Cirik est transférée dans *Paradoxia (P. paradoxioides* n. comb.). La distinction entre les genres *Paradoxia* et *Ankyra* devient un peu plus claire : aptitude à produire des cénobes, comportement de la paroi mère lors de la sporulation, présence de soies, morphologie du pyrénoïde et forme des pôles cellulaires. Le cas de *Actinastrum guttula* Playfair (= *Paradoxia multiseta* ?) est discuté.

KEY WORDS : *Paradoxia, Ankyra, Actinastrum*, zoospores, coenobia, life cycle, taxonomy.

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INTRODUCTION

Although *Paradoxia multiseta* Svirenko (1928) has been observed world wide, it is always rare and therefore, little is known about the life cycle. Nevertheless, some hypotheses have been developed. FOTT (1953) proposed that the coenobium was formed through the junction, but not the copulation, of two free swimming zoospores, which then form the typical leaf-like anterior appendages. BOURRELLY (1966) suggested that according the hypothesis of FOTT (1953), *Paradoxia* should be placed in the Hydrodictyaceae. WAWRIK (1977) observed that the protoplast of one cell of *Paradoxia* divided in four parts, while CIRIK (1978) saw the division in two parts. REYMOND & DRUART (1982) supposed, after observing a few EM micrographs of rare field material, that zoospores were formed. They hypothetised that the flagella did not function, because of the very early formation of the coenobium inside the mother cell wall.

In addition to the above mentioned hypothesis directly concerning the life cycle of *Paradoxia*, REYMOND (1979) established that the ultrastructure of the anterior leaf-like appendages of *Paradoxia multiseta* is similar to that of species of *Ankyra*. From his observation it was proposed that these two genera are more closely related than previously thought, and their life cycle might be comparable. *Ankyra* is a frequently encountered alga. SWALE & BELCHER (1971) who studied the ultrastructure and the life cycle of a strain of *Ankyra*, also observed zoospores. Later, FOTT (1974) observed the formation of resting stages (cysts) in *A. judayi* (G.M. Smith) Fott. KRIENITZ & HEYNIG (1982) made the same observation in *Ankyra lanceolata* (Kors.) Fott (= *A. spatulifera* (Kors.) Fott). According to this literature review, our knowledge of the life cycle of *Paradoxa multiseta* is incomplete. An attempt to culture *Paradoxia* failed and only one young vegetative cell was briefly observed (REYMOND, 1979). More recently a strain was successfully isolated by the first author, and its life cycle was observed and briefly discussed (HEGEWALD & REYMOND, 1985). The main ultrastructural features of the adult vegetative cells and cysts were previously published by REYMOND & HEGEWALD (1987). Our present purpose is to show and discuss more extensively than before the life cycle of *Paradoxia multiseta* and reconsider the taxonomic position of some related species.

MATERIAL AND METHODS

A unialgal strain of *Paradoxia multiseta* was isolated in 1982 from the reservoir «Haltener Stausee» (FRG) and later deposited at the Algae Collection of the University of Göttingen (FRG) as SAG B 18.84 (SCHLÖSSER, 1984) and the Culture Collection of Algae at the University of Texas at Austin (USA) as UTEX 2460 (STARR & ZEIKUS, 1987). Most observations were made with light microscopy. The techniques used for EM were published in REYMOND & HEGEWALD (1986).

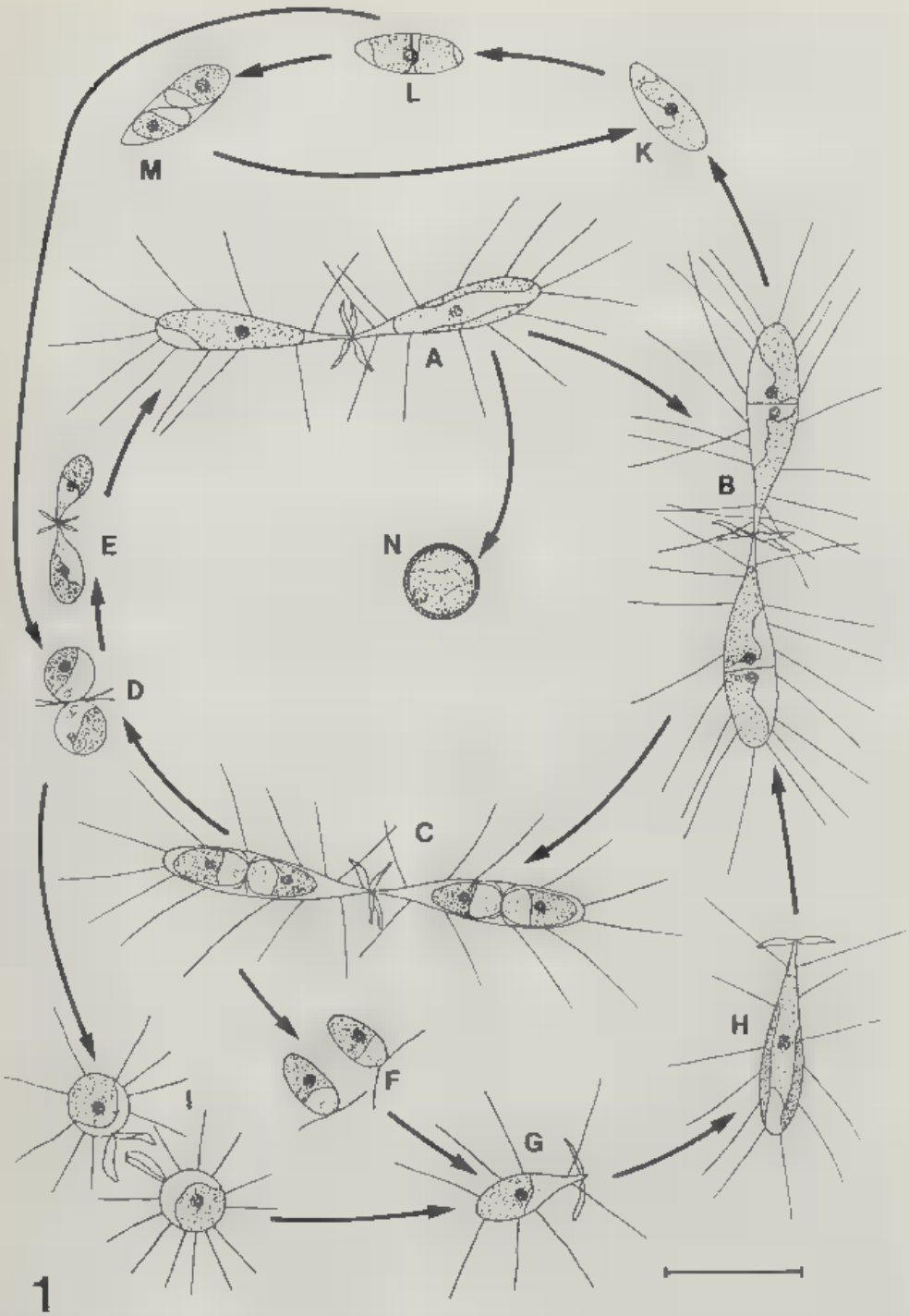
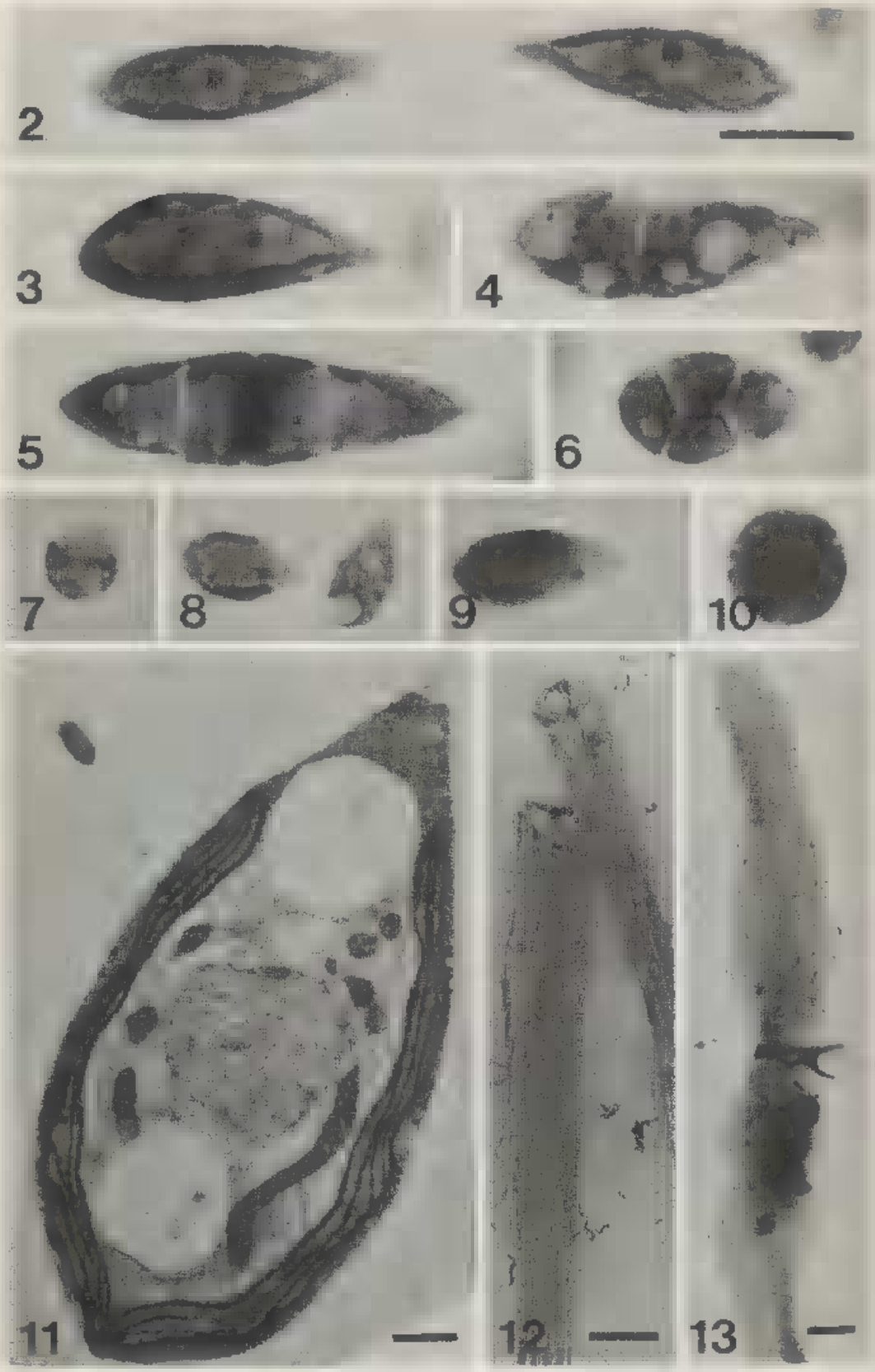


Fig. 1 – The life cycle of *Paradoxia multiseta*. A-E, typical cycle including only coenobial stages. C, F-H, B, cycle with formation of free zoospores. D, I, G, H, B, cycle including the separation of coenobial cells after their release from the mother cell. K-M, autospore formation. A, N, cycle including the formation of a resting stage (cyst). Its germination has not yet been observed. Bar : 10 μm.



RESULTS

Within the mother cell wall, two (Fig. 1B, 1C, 3, 4), four (Fig. 5, 6) or sometimes more daughter cells are formed which are released in seconds by a quick dissolution of the cell wall. The only remnants of the mother cell wall found with light or electron microscopy are the anterior tips of the cells, with their leaf-like appendages (Fig. 13). The daughter cells are released in joined pairs (Fig. 1D) or individually as zoospores (Fig. 1F). The former cells (Fig. 1D) are attached at their anterior tips and swim for about a minute, after which the flagella disappear and the typical leaf-like appendages are formed (Fig. 1E, 12). Because of the curvature of cells in the attachment area, no details of flagella loss were observed. Shortly after their release, the daughter cells usually produce bristles. Two contractile vacuoles remain active long after the disappearance of the flagella but are not present in adult cells. A stigma is always present in zoospores and vegetative cells at the EM level but is not always visible with light microscopy. On rare occasions, the cells of the young coenobia separate and become normally developed vegetative cells (Fig. 1I, 1G). When free, biflagellate zoospores are released from the mother cell wall, they swim for a long time, then their flagella disappear and leaf-like appendages are formed. The resulting unpaired vegetative cells are identical to each of the paired vegetative cells (Fig. 1F, 1G). We have never observed free swimming cells coming in contact with each other to produce paired cells and for this reason it is unlikely that coenobia are formed by the aggregation of free swimming cells. Occasionally the vegetative cells form resting stages (cysts), each with a thick wall (Fig. 1N, 10). Germination of the cysts has not yet been observed. The occasional appearance of zoospores with 4 (Fig. 14) or more flagella is probably the result of abnormal development. The abnormal zoospores have flagella which are arranged in sometimes widely separated pairs. They also have a larger cell

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Fig. 2-10. *Paradoxia multiseta*. Bright field light microscope micrographs of Spurr resin flat embedded cells. Cell to cell junction, flagella or leaf-like appendages are not visible here. Bar: 10 µm. Figs 2 to 10 are the same scale. Fig. 2 : Two vegetative cells constituting the coenobium. Fig. 3 : Cell with two nuclei. Fig. 4 : Mother cell divided into two daughter cells. Fig. 5 : Mother cell divided into four daughter cells (typical arrangement). Fig. 6 : Mother cell divided into four daughter cells (untypical arrangement). Fig. 7 : Zoospore. Fig. 8 : Young vegetative cell just after the disappearance of the flagella. Fig. 9 : Young vegetative cell a few hours after its formation. Fig. 10 : Cyst. Fig. 11 : Electron micrograph of a section. Young vegetative cell with nucleus, chloroplast, two huge vacuoles and several profile of one (or eventually two) mitochondrion. Bar : 1 µm. Fig. 12, 13, Electron micrographs of material deposited on formvar coated grids. Fig. 12 : Detail of the distal part of two young and still enrolled leaf-like appendages at the apical pole of a young vegetative cell after the disappearance of the flagella (the cell body is not visible here). It is not always possible to distinguish between flagella and forming appendages with the light microscope. Therefore the short transition period between flagella disappearance and appendage formation is not yet fully documented. Fig. 13 : Remnants of the mother cell wall after its dissolution : apical part of the mother cell wall with its leaf-like appendages. Bar : 1 µm.

volume than normal and two chloroplasts. Because cell fusion was never observed, we suggest that these are the result of incomplete cell division. However, they apparently are able to develop into vegetative cells, i.e., few cells are observed with two apical cell poles, both with leaf-like appendages (Fig. 15), which also indicates incomplete cell division. These cells also have two pyrenoids.

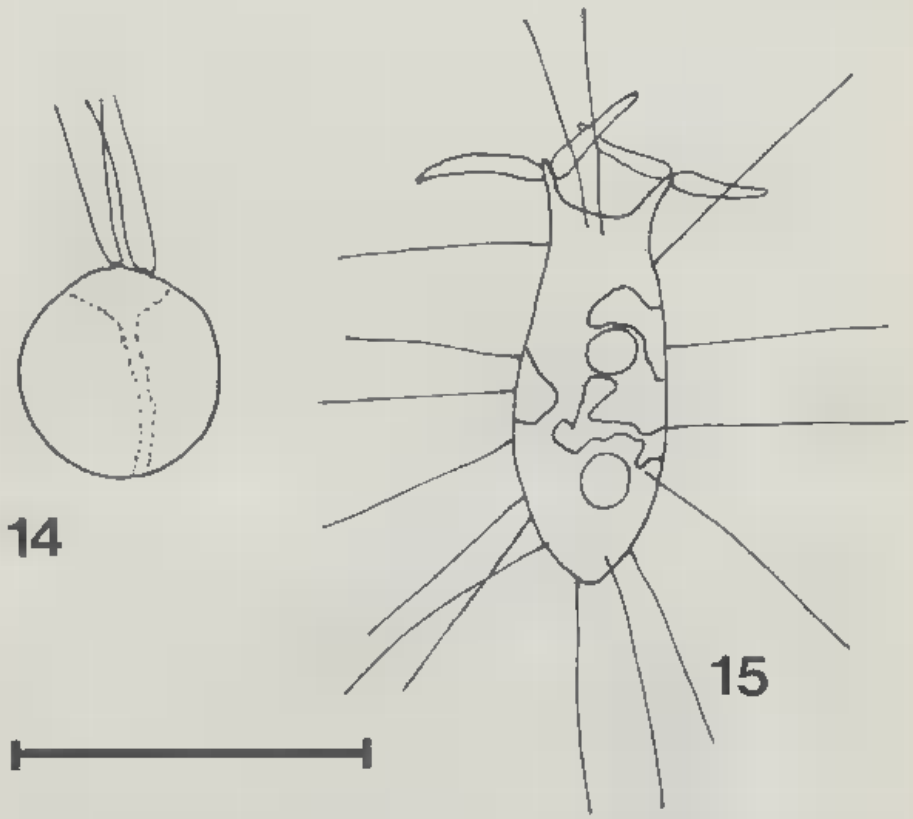

Fig. 14, 15. *Paradoxia multiseta*. Bar : 10 µm. Fig. 14. Abnormal zoospore with four flagella. Fig. 15 . Abnormal vegetative cell with divided apical pole.

In older cultures, possibly due to nutrient limitation, autospores are exclusively produced (Fig. 1K). They are very unlike the other vegetative cells in appearance having neither bristles nor apical appendages. And the apical cell pole is broadly blunt and not elongated or only slightly. The autospores are also very small, usually less than 10 µm long. In the mother cell autospore production is preceded by one or two divisions of the chloroplast (Fig. 1L). After the autospores are formed they remain within the mother cell wall (Fig. 1M), apparently for hours. The remnants of the mother cell wall remain visible in the media unlike those from zoospore production, although they are finally dissolved. Autospores can also produce paired (or single) zoospores (Fig. 1L, 1D), but we do not know the conditions of their induction.

DISCUSSION

From our observations we conclude that none of the earlier hypotheses concerning the life cycle of *Paradoxia multiseta* are fully correct. It is now accepted (KOMAREK & FOTT, 1983) that *Paradoxia* and *Ankyra* are taxonomically related as proposed by REYMOND & DRUART (1982). In *Ankyra*, SWALE & BELCHER (1971) found that the mother cell wall either breaks transversally into two parts or a hole is formed in the side of the center; in each case the empty cell wall remains visible for a long time. With another strain of *Ankyra* (Hegewald 1985-1) we also observed the mother cell wall breaking into two parts when releasing spores. Differences in the break down of the mother cell wall during sporulation offer a relatively good means of distinguishing these two genera. It is not yet absolutely clear whether the zoospores inside the mother cell wall are briefly free before the coenobia form (as for exemple in *Pediastrum*), or whether they are never fully separated during their formation. In any case this kind of coenobium differs from that in all other Chlorococcales and Volvocales by the position of the cell junction. Although we might expect sexuality from the two paired cells, similar to the copulation in Volvocales, it was never observed. In addition to these observations, REYMOND & HEGEWALD (1986) found (with one exception) obvious differences of pyrenoid ultrastructure between *Paradoxia* and *Ankyra*. So both genera are little bit better separated than before (REYMOND & DRUART 1980; KOMAREK & FOTT, 1983), even if actually none of the diacritical characters are absolute. *Paradoxia* has bristles which are missing in *Ankyra*. *Paradoxia* in nature usually and in culture often, has paired cells with a blunt basal cell pole, while *Ankyra* is always unicellular and has an acute basal cell pole (The leaf-like appendages are in *Ankyra* and *Paradoxia* at the apical pole, not at the basal cell pole as described by KOMAREK & FOTT (1983)).

Due to the relative value of the diverse characters belonging to *Paradoxia* and *Ankyra*, the place of some taxa can be a problem to the taxonomists :

Paradoxia pelletieri, though unicellular is clearly not an *Ankyra*, as proposed in KOMAREK & FOTT (1983). In addition, new observations of this species in Lake Léman (1986) by DRUART (personal communication) confirms that it is a separate species and not a single celled *Paradoxia multiseta*.

Ankyra paradoxioides Cirik is similar to *P. multiseta* in that it also has bristles and a blunt basal cell pole which is less obtuse. Although we do not know whether the mother cell wall dissolves or breaks in two parts prior to sporulation, there is no doubt that the species belongs to the genus *Paradoxia*. It differs from *P. multiseta* in having a less blunt basal cell-pole, two thicker bristles below the apical pole and 2-3 at the basal pole :

Paradoxia paradoxioides (Cirik) Hegew. et Reymond.
Basionym : *Ankyra paradoxioides* CIRIK (1978), *Rev. Algol.*, N.S. 13 : 210.

Ankyra calcarifera (Kisel.) Fott has a single spine below the apical pole, which may prove to be similar to the bristles of *Paradoxia*. However, it is doubt-

lessly an *Ankyra*, by virtue of its very acute elongated basal pole and splitting of cell walls in sporulation. A problem arises with *Ankyra inerme* Reymond & Druart, which shows a breaking of the mother cell wall (unpubl.) and the lack of bristles (typical of *Ankyra*), but also horsetail-like appendages (unpubl.) and a blunt basal cell pole more typical of *Paradoxia*. In addition to this taxonomical discussion, the coenobial alga *Actinastrum guttula* was not described clearly by PLAYFAIR (1916) but is certainly representative of *Paradoxia*, as previously stated by FOTT (1953) and KOMAREK & FOTT (1983). Despite sharp details in the drawing of other taxa in the same plate, the poor representation of the anterior leaf-like appendages of the coenobium of *Actinastrum guttula* make us believe that details like the bristles, which are sometimes very fine or sparse (rarely absent, KOMAREK, 1974) were probably overlooked by bright field microscopical observations, rather than missing. We studied the type sample for *A. guttula*, but could find only few algae and no *Paradoxia*. A synonym between *Paradoxia multiseta* and *Actinastrum guttula* is very likely. However without much more detailed information concerning Playfair's material (or really identical material) this decision would be premature and *Actinastrum guttula* must be considered as a *nomen dubium*.

ACKNOWLEDGEMENTS

We thank Prof. B. Droz and Mr. P.A. Milliquet for the EM facilities, Mrs G. Groehn for help in culturing and Dr. J. Zeikus for his conscientious correction of the language. We also thank Mrs. A.-M. Philippe for her help in the typing of the manuscript. We are grateful to Prof. P. Bourrelly and Dr. H.R. Preisig for their scientific attention during the corrections of the final manuscript.

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